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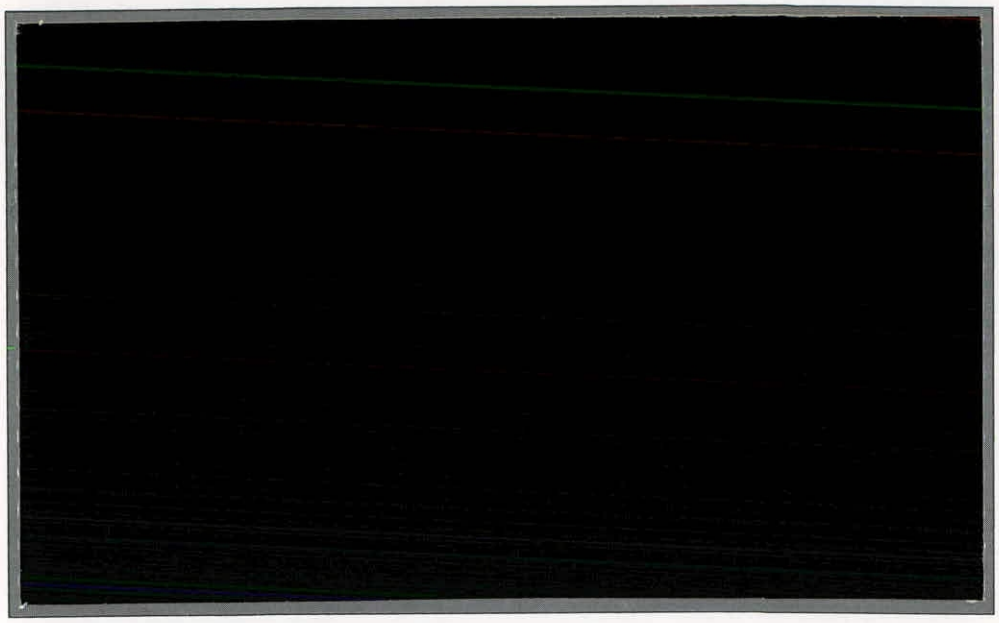

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Natural
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INSTITUTE OF FRESHWATER ECOLOGY
River Laboratory, East Stoke, Wareham, Dorset BH20 6BB

Tel: 0929 462314
Fax: 0929 462180

Preparation, reconstitution and homogeneity studies of lyophilised permethrin and simazine containing water samples

FIRST INVESTIGATIONS OF THE STABILITY STUDY

W.A. House PhD FRSC C.Chem and D.R. Orr

Project leader: W.A. House
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Abstract

A 24 kg batch of freeze-dried river water containing the pesticides permethrin and simazine has been prepared. A methanol extract from 5 litres of river water has been prepared and stored at -20°C.

The results of the reconstitution and glc analysis of triplicate 1 litre samples with blank are presented for the reference time at the start of the stability trial and after 1 months storage. The results show that simazine is stable in the freeze-dried solid and that further study is necessary to assess the stability of permethrin. There is some evidence that permethrin is degrading during storage but this needs to be confirmed after a total of 3 months storage.

1. Preparation of bulk river water sample

A fifty litre quantity of river water was collected from the R. Frome at the East Stoke weir (National Grid Reference SY 868868) at 14.00 hours on 5 January 1990. This was stored in a 60 litre polypropylene container prior to sampling 1 litre quantities for freeze-drying. The pH, temperature and conductivity of the river water was measured immediately after collection.

River water from the 50 litre sample was filtered through a 0.45 μm cellulose nitrate membrane filter (Sartorius 11306 No. 7802119604209) into a 1 litre pyrex bottle with PTFE screw cap and stored at 5 ^{o}C in the dark. The first litre of filtered water was analysed for major-ions and nutrients and the results are shown in Table 1. Subsequent 1 litre samples, labelled BCR2-30, were filtered and stored in the same way.

2. Preparation of freeze-dried samples

On 10th January 1990, the river water samples were allowed to return to room temperature before being spiked with the pesticides cis-permethrin and simazine. This was done by the addition of 1 ml of 52.2 mg dm^{-3} simazine and 1 ml of 4.99 mg dm^{-3} cis-permethrin dissolved in acetone and shaking for about 2 minutes. The concentration of simazine and permethrin in the river water was 0.052 mg dm^{-3} and 4.99 μg dm^{-3} respectively. Five of the 1 litre samples were selected as blanks ie numbers 2, 9, 16, 23 and 30 (see Table 2 for bottle coding). A further 5 bottles (numbers 4, 12, 19, 26, 28) were selected for immediate extraction and designated as the 'raw' extract samples. The remaining 19 spiked samples and 5 blank samples were then transported in the 1 litre pyrex bottles for freezing and freeze-drying. The transportation took approximately 2$\frac{1}{2}$ hours. On arrival, the samples were transferred to aluminium trays and then frozen immediately. The freeze-drying was accomplished over a period of 7 days. The freeze-dried samples were returned for the stability study on 23 January 1990.

The pesticide spiked freeze-dried solids were transferred from the trays to a single 250 ml pyrex bottle. Similarly, the 5 blank samples were bulked together with every precaution to ensure that no cross-contamination occurred with the pesticide spiked samples. The content of each tray was weighed on a 4 decimal, top-pan balance (Mettler model AE200). The results are shown in Table 2. The bulked freeze-dried samples (spiked and blank) were then thoroughly shaken to homogenise the solids.

0.3394 $\pm$ 0.00039 (SD, n=18) subsamples of the freeze-dried spiked solids were weighed into 1 litre pyrex glass bottles and coded as previously. Similarly, 5 sub-samples of the blank material were weighed and coded to give a mass of 0.3393 $\pm$ 0.0002 g (SD, n=4). the individual weights of all the sub-samples are shown in table 2. The percentage variability in the sub-sample weights was <0.1%. All the samples were stored at room temperature in a nitrogen gas atmosphere and in the dark.

3. Reconstitution of the freeze-dried samples

Three spiked samples and one blank sample were selected at random at the beginning of the stability trial on 31 January 1990. This date will be subsequently referred to as the reference time. On 5 March 1990, 32 days from the reference time, a further 4 samples including a blank, were selected at random. Both series of samples were reconstituted and extracted as quickly as possible after selection using the procedure determined in the pilot study (report to BCR: IFE RL/T04053o1/1, November 1989). In brief the reconstitution procedure was as follows:

3.1 1 litre of single distilled water was added to the freeze-dried solid in the 1 litre pyrex bottle used for storage. A PTFE magnetic bar was added.

3.2 A stream of CO_2 gas was passed through the solution at a rate of ≈60 ml min^{-1} for a period of 110 minutes whilst the solution was stirred.

3.3 The CO_2 gas was turned off and approximately 75 mls of solution removed for conductance and temperature measurement (pH ≈ 5.1).

3.4 Nitrogen gas was then bubbled through the solution at a rate of 1 l min^{-1} for 15 minutes. After this time 100 mls of solution were removed for the measurement of the final pH (see Table 3).

The codes for the samples selected at the reference time and after 32 days together with the final pH and conductivities corrected to 25 °C are shown in Table 3. Two of the samples, BCR9 and 23, were analysed for major-ions and nutrients (see Table 1). The percentage recoveries calculated from the conductivity of the original R. Frome water and measurements on the reconstituted samples are shown in Table 3.

The results in Table 1 show that the measurement of conductivity (after correction to 25 °C) is an excellent method of assessing the overall recovery of the major-ions. For the 8 samples so far reconstituted in the stability study, the mean recovery estimated from conductivity measurements is 84.1% with a percentage variation of <0.4% (see Table 3).

4. Extraction procedure

Simazine and permethrin were extracted together using solid-phase extraction techniques (SPE). A Bond-Elut adsorption column containing octyl (C8) bonded phase silica as the adsorbent (Analytichem International Code P606303) was used. The method was as follows.

4.1 The C8 column was fitted to a Vac-Elut (Analytichem International, model AI6000) assembled and washed with 2-3 ml of HPLC grade MeOH. The column was left for 5 minutes in contact with MeOH. The column was not allowed to become dry.

4.2 Approximately 15 ml of HPLC grade water was passed through the column.

4.3 The column was then transferred to the entrance of a 250 ml capacity polycarbonate reservoir which could be evacuated as required. The top of the column was connected to the sample to be analysed using small bore PTFE tubing. The sample in the 1 litre bottle was stirred continuously using a PTFE magnetic bar and motor.

4.4 Approximately 250 ml of sample was then passed through the column at a rate of ≃ 5 ml min^{-1}. The volume which had passed through the column was calculated from the weight of water in the reservoir with appropriate buoyancy correction.

4.5 The column was washed with 20 ml of HPLC grade water and dried for ≃ 20 minutes at maximum air flow.

4.6 The column was then transferred to the Vac-Elut assembly and eluted with ≃ 2 ml of MeOH. The volume of eluate was calculated from the change in mass of the collecting vial and assuming a density of MeOH of 0.7910 g ml^{-1}. Precautions were taken to avoid evaporation of MeOH during and after weighing.

4.7 Extracts were stored in 4 ml PTFE screw-capped glass vials at 6°C before analysis by glc.

5. GLC analysis

The extracts were analysed using a Perkin-Elmer glc model 8700 fitted with a split/splitless injector ECD detector and PTV injector - NPD detector.

5.1 Analysis of simazine

Configuration

Injector	-	PTV	(Programmable Temperature Vaporizer)
Column	-	DB1301	Jones Chromatography
Detector	-	NPD	(Nitrogen Specific Detector)

Oven conditions:	Initial temperature	140°C for 1 minute
	First ramp	20°C per minute
	Isothermal	240°C for 7 minutes

Injector conditions:	Initial temperature	100°C for 1 minute in split mode
	Vaporization temperature	280°C for 5 minutes in splitless mode for 1 minute
	Final temperature	150°C

Gases:	Makeup:	N_2
	Carrier:	He
	Septum purge:	≃ 5 ml min^{-1}
	Flow rate:	≃ 50 ml min^{-1}

Analysis by external standard mixture of 5 mg dm^{-3} Simazine and 0.5 mg dm^{-3} cis-permethrin.

5.2 Analysis of Permethrin

Configuration:

Injector	-	Split/splitless	
Column	-	DB5	Jones Chromatography
Detector	-	ECD	(Electron Capture Detector)

Oven conditions:

Oven temperature (°C)	50	170	240	280
Isothermal time (min)	2.0	0.0	7	2
Ramp rate (°C min^{-1})	30.0	10.0	2.0	

Injector conditions:

Temperature 310°C
Splitless for 30 seconds

Detector condition:

Temperature 350°C

Gases: Makeup: N_2
Carrier: He
Septum purge ≃ 5 ml min^{-1}
Flow rate ≃ 50 ml min^{-1}

Analysis by external standard mixture of 5 mg dm^{-3} simazine and 0.5 mg dm^{-3} cis-permethrin.

Due to instrument failures involving the split/splitless injector the reference time analysis of permethrin was done using the PTV injector and ECD configuration.

6. Preparation of the raw extract

On 10th January 1990, five samples of R. Frome water, bottle numbers 4, 12, 19, 26 and 28, were spiked with permethrin and simazine as described in §2. These samples were shaken for 15 minutes and then extracted using the solid-phase-extraction method described above in §4. A total of 9.93 mls of extract in methanol were isolated from 1208 ml of water from the 5 samples. This combined extract, referred to as the raw extract, was stored in the dark at -20°C.

7. Results of the pesticide analysis

7.1 Permethrin stability

The results of the analysis are shown in table 4. The reference time analysis of permethrin gave a concentration of 3.87 ± 0.29 (SD) $\mu g\ dm^{-3}$. This corresponds to a permethrin recovery in the freeze-dried samples of 78% for permethrin. The standard deviations are similar to those obtained in the pilot study.

After 32 days storage, the concentration of permethrin was determined as 3.09 ± 0.39 (SD) $\mu g\ dm^{-3}$ which is significantly (t-test, 5%) different from the reference time analysis but not significantly different from the concentration (2.88 $\mu g\ dm^{-3}$) found in the raw extract (t-test, 5%). An example of the chromatograms obtained for the reconstituted samples and raw extracts are shown in Figure 1.

7.2 Simazine stability

The results of the analysis are shown in Table 5. The reference time analysis of simazine gave a concentraiton of 0.040 ± 0.002 (SD) mg dm^{-3} corresponding to a recovery in the freeze-dried sample of 77%.

After 32 days storage, the concentration of simazine in the reconstituted sample was determined as 0.047 ± 0.004 (SD) mg dm^{-3} which is significantly different from the reference time analysis (t-test, 5%) but within agreement with the analysis of the raw extract (t-test, 5%). This indicates that there is no significant loss of simazine in the freeze-dried samples although the reason for the higher concentration in the stored samples is not known. Sample extracts are being stored to permit tests for degradation products which may be co-eluting with simazine. This is considered to be unlikely, particularly because of the agreement between the analysis of the raw extract and the samples from the second reconstitution.

Examples of the chromatograms obtained for the analysis of the raw extracts and reconstituted samples are shown in Figure 2.

8. Conclusion

The bulk freeze-drying and reconstitution procedures have proved to be successful. The reconstitution method has proven particularly reliable and has produced consistent recoveries of 84.1%. The measurement of the electrical conductivity corrected to 25°C produces an excellent method of checking the efficiency of the reconstitution procedure.

The concentration of permethrin measured in the reconstituted samples after 1 months storage was not significantly different from that in the raw samples which had been stored in methanol at -20°C. Further study is necessary to assess any deterioration in the freeze-dried solid.

The results for simazine are very encouraging. They show that simazine in the freeze-dried samples is stable for a period of at least 1 month.

Table 1. Comparison of R. Frome water and reconstituted waters

	Conductivity† at 25°C /µS cm^{-1}	pH	Alkalinity meq dm^{-3}	Na^+	K^+	Ca^{2+}	Mg^{2+}	SO_4^{2-}	Cl^-	NO_3^-	SiO_2	PO_4 /µmol dm^{-3}
							/mmol dm^{-3}					
R. Frome 5.1.90	552.1	7.95	4.09	0.51	0.03	2.50	0.10	0.57	0.69	0.44	0.07	4.9
BCR 9 5.2.90	462.5	8.14	3.50	0.38	0.04	2.07	0.09	0.48	0.48	0.39	0.06	2.6
% Recovery	84		86	75		83	90	84	70	89	86	53
			Mean recovery of major-ions listed is 83 ± 7% (SD)*									
BCR 23 5.3.90	463.6	7.96	3.70	0.40	0.05	2.04	0.08	0.49	0.51	0.39	0.06	4.1
% Recovery	84		91	78		82	80	86	74	89	86	84
			Mean recovery of major-ions listed is 83 ± 5% (SD)									

† corrected to 25°C according to the method of Wagner (1971)

* excludes PO_4

Table 2. Sample codes, assignments and freeze-dried powder weights

BCR Number	Assignment	Mass of freeze-dried solid /g	Mass of sub-sample after homogenization/g
1	Inorganic Analysis	-	-
2	Blank	0.3321	0.3393
3	X	0.3092	0.3393
4	Raw	-	-
5	X	0.3351	0.3395
6	X	0.3503	0.3391
7	X	0.3361	0.3395
8	X	0.3222	0.3391
9	Blank	0.3465	0.3390
10	X	0.3686	0.3393
11	X	0.3548	0.3393
12	Raw	-	-
13	X	0.3514	0.3398
14	X	0.3595	0.3391
15	X	0.3135	0.3395
16	Blank	0.3363	0.3394
17	X	0.3242	0.3395
18	X	0.3580	0.3393
19	Raw	-	-
20	X	0.3435	0.3384
21	X	0.3404	0.3397
22	X	0.3390	0.3396
23	Blank	0.3480	0.3394
24	X	0.3494	0.3340
25	X	0.3460	0.3396
26	Raw	-	-
27	X	0.3575	0.3392
28	Raw	-	-
29	X	0.3331	0.3396
30	Blank	0.3599	0.3148*
		Mean 0.3417 ± (0.0161 SD)	**Mean** 0.3393 ± (0.0003 SD)

Key Blank: no pesticide spike
Raw: not freeze-dried; extracted after spiking
X: pesticide spiked sample
-: not applicable

Footnote * not included in the calculation of the mean

Table 3. Conductivity of the reconstituted water samples measured at pH ≈5.1 and corrected to 25 °C

Sample Number BCR #	Final pH	Conductivity /μS cm^{-1}	Percentage Recovery
	Results at the Reference time		
3	6.7	466.8	84.5
17	7.9	460.9	83.5
22	7.8	465.8	84.4
Blank 9	8.1	462.5	83.8
			Mean 84.1 ± 0.5 (SD)
	Results after 32 days		
6	8.0	464.4	84.1
14	7.8	464.9	84.2
24	8.0	464.8	84.2
Blank 23	8.0	464.9	84.2
			Mean 84.2 ± 0.05 (SD)

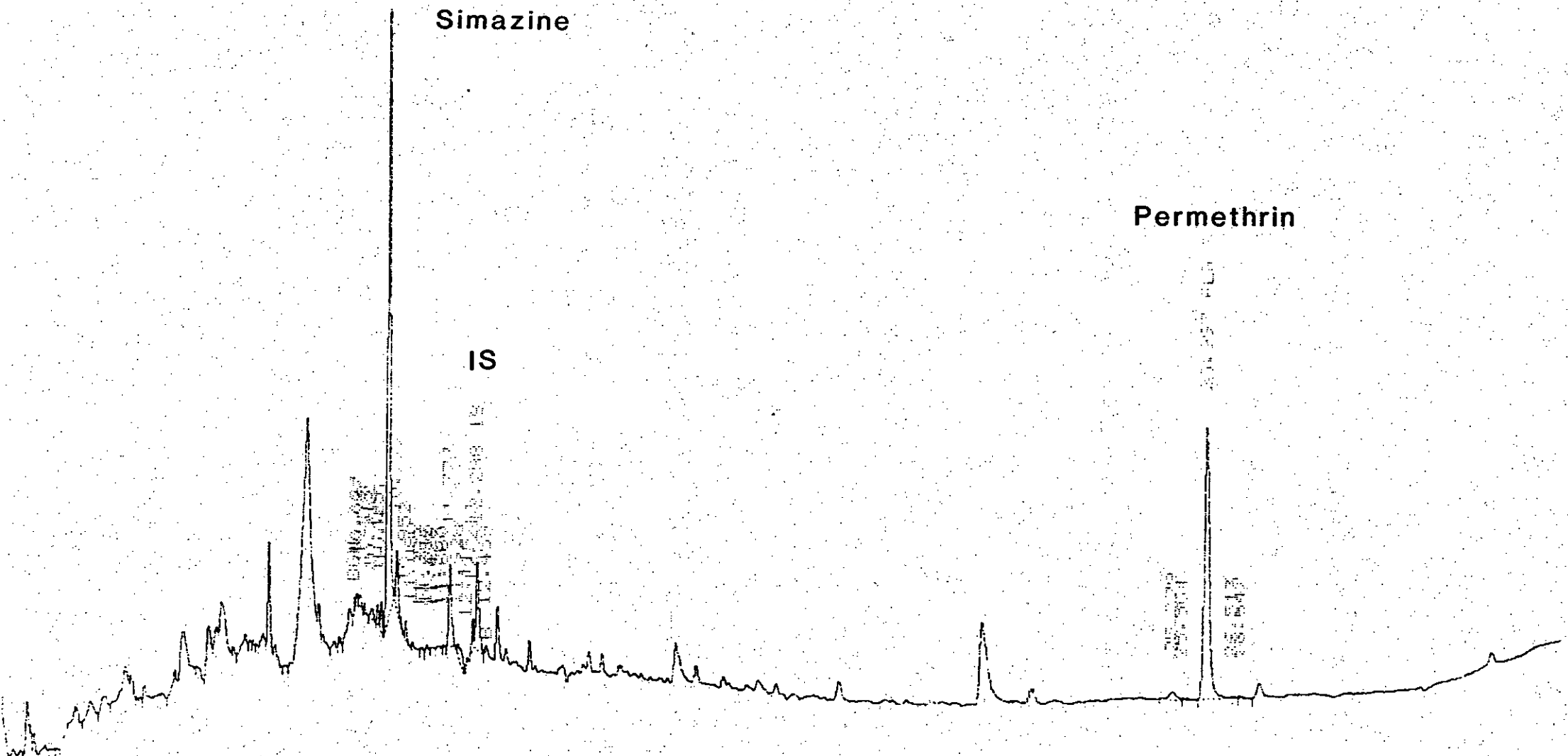
Table 4. Results of Permethrin analysis

Sample Designation	Storage Interval/d	Concentration in Water/$\mu g\ dm^{-3}$	Standard deviation	Number Replicates
BCR 6	0	3.67	0.25	3
BCR 17	0	4.16	0.13	2
BCR 22	0	3.89	-	1
Mean		**3.87**	**0.29**	**6**
BCR 6	32	2.92	0.30	3
BCR 14	32	3.19	0.39	3
BCR 24	32	3.15	0.55	3
Mean		**3.09**	**0.39**	**9**
Raw	32	2.88	0.12	3

No Permethrin detected in the blank samples

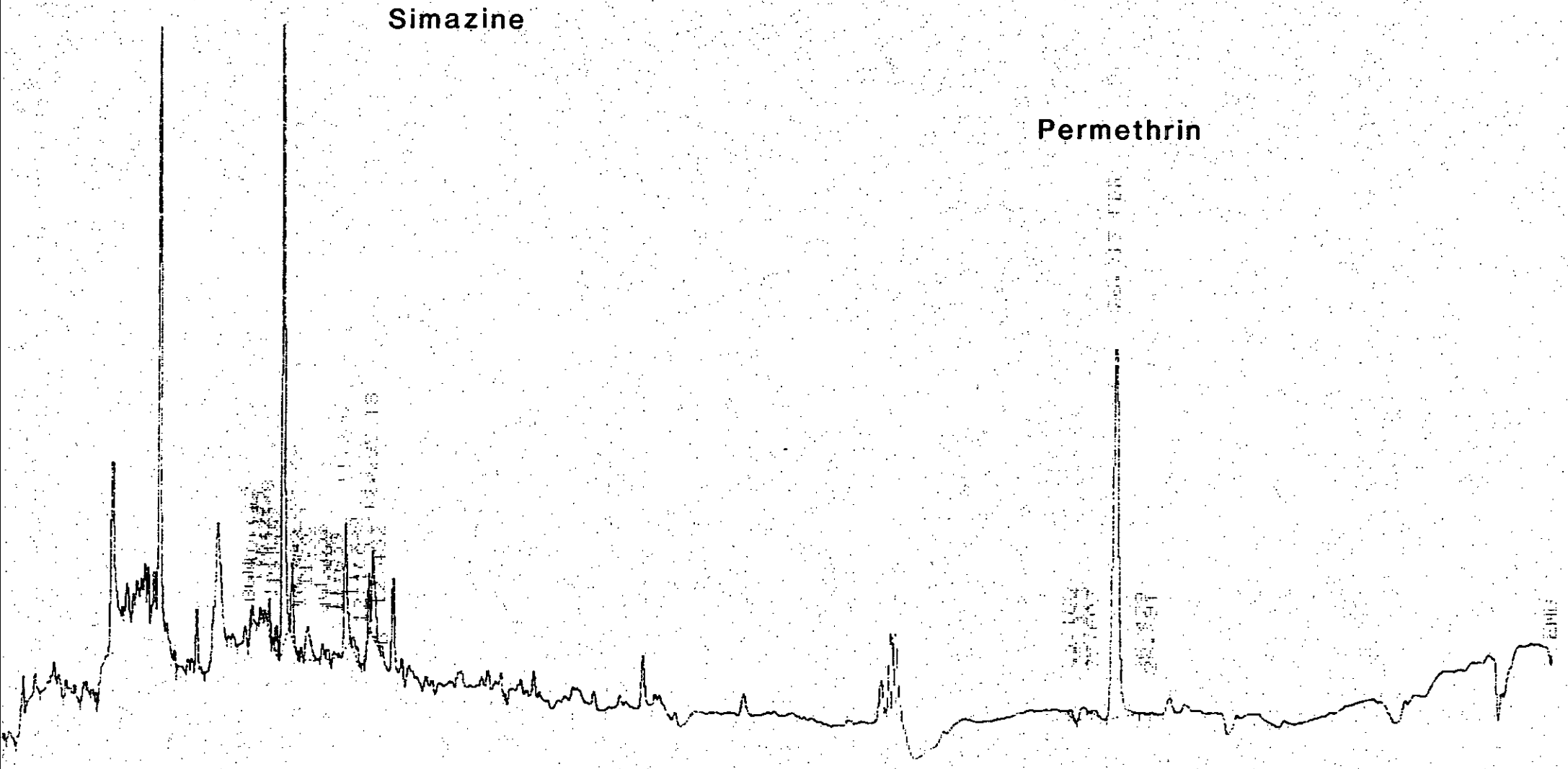
Table 5. Results of Simazine analysis

Sample Designation	Storage Interval/d	Concentration in Water/$\mu g\ dm^{-3}$	Standard deviation	Number Replicates
BCR 3	0	0.041	0.001	2
BCR 17	0	0.042	0.0004	3
BCR 22	0	0.037	0.001	3
Mean		**0.040**	**0.002**	**8**
Blank	0	0.002	0.002	3
BCR 6	32	0.047	0.005	3
BCR 14	32	0.046	0.005	3
BCR 24	32	0.048	0.001	3
Mean		**0.047**	**0.004**	**9**
Blank	32	Not detected		
Raw	32	0.042	0.005	3



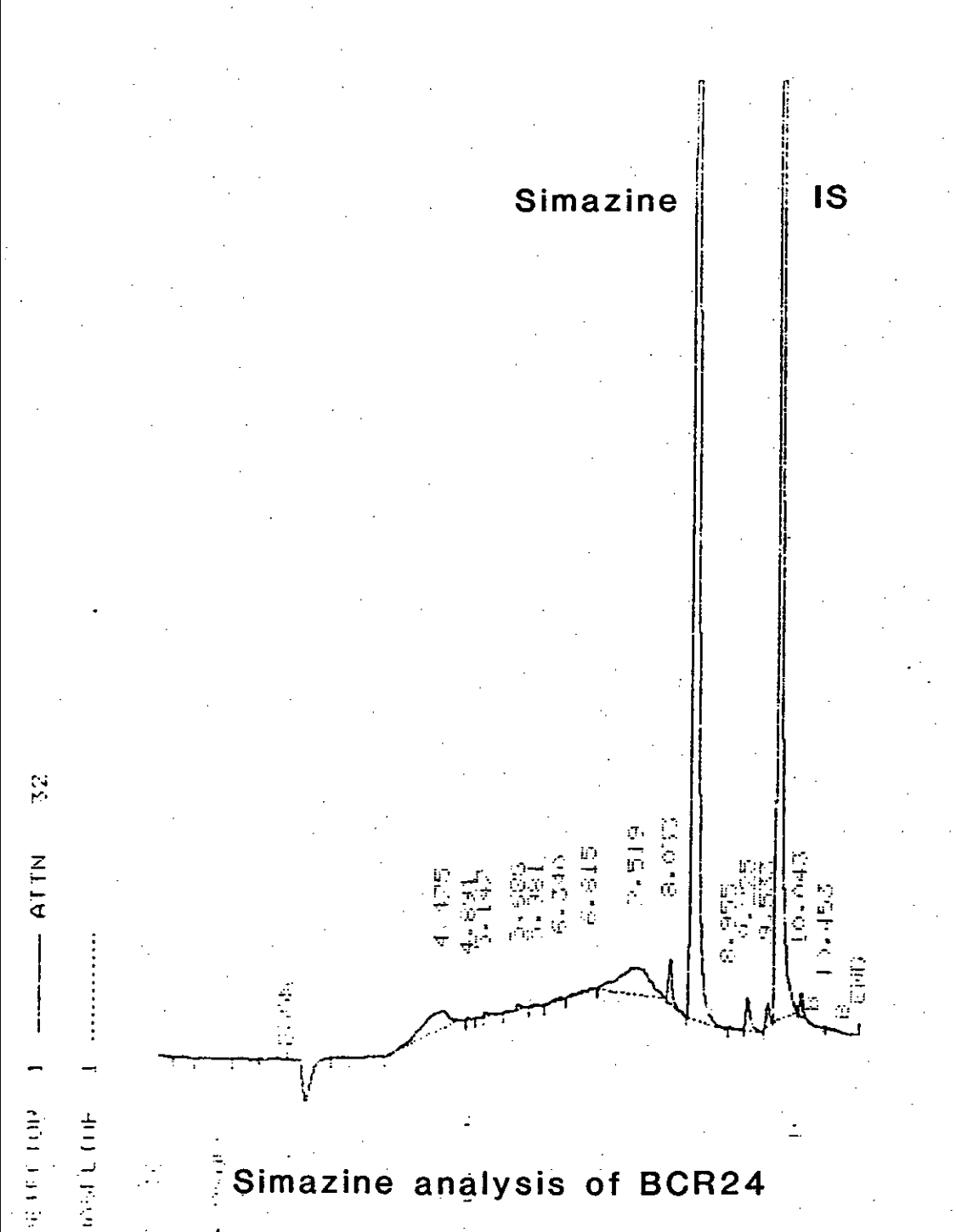
RAW EXTRACT

FIGURE 1a

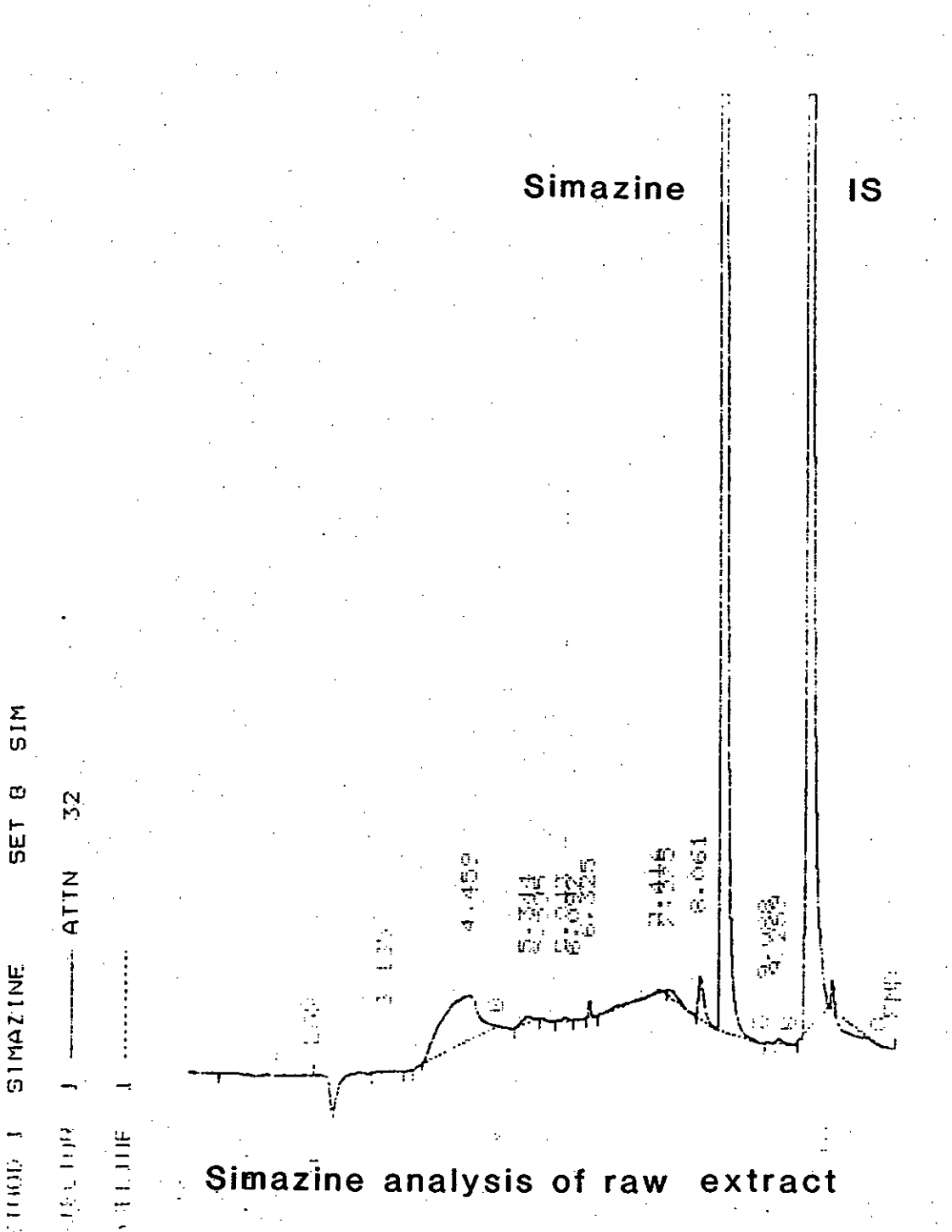


BCR 24

FIGURE 1b



Simazine analysis of BCR24



Simazine analysis of raw extract

FIGURE 2